# Applying the Mesolens to Microbiology

## Visualising Biofilm Architecture and Substructure

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## Introduction

Biofilms pose an increasing public health risk due to their ability to confer chemical, mechanical and environmental protection to the constituent bacteria[1]. Previous studies have shown complex fractal patterning and chirality in multi-strain colony biofilms; however, the architecture and substructure of single-strain communities is somewhat understudies. **We aim to use the Mesolens to image the previously unexplored internal architecture of an intact colony biofilm to better understand spatiotemporal organisation of a live bacterial community.**

The Mesolens is a large objective lens with a low magnification (x4) lens capable of imaging a large field of view (6*x*6 $mm$) with a 3 $mm$ working distance in either widefield epi-fluorescence or laser scanning confocal modes. A high numerical aperture (N.A. = 0.47) results in lateral resolution of 700 $nm$ and axial resolution of 7 $\mu m$ [2]. The Mesolens allows for imaging relatively large samples with sub-cellular resolution throughout the dataset with no change in objective magnification.

Using the Mesolens we have observed the internal architecture of *Escherichia coli* colony biofilms and documented previously unreported channel systems. We hypothesise that these channels are involved in structural support and nutrient dissemination throughout the biofilm.

## Methods

**Strains & Culturing;**
*Escherichia coli* (JM105) expressing EGFP were inoculated at a density of 1x10$^8$ cfu/$ml$ on LB medium ([Gentamicin] = 20 $\mu g/ml$) and incubated for 18 hours at 37°C in custom 3D-printed imaging moulds. Specimens were mounted in sterile LB broth prior to imaging.

**Widefield Epi-fluorescence and Confocal Laser Scanning Mesoscopy;**
For widefield epi-fluorescence imaging, excitation light was sourced from a pE-4000 LED lightsource (CoolLED, U.K.) at the appropriate wavelength ($\lambda_{ex.}$ = 490 $nm$) and emission was detected via a CCD camera detector (Stemmer Imaging, U.K.). For laser scanning confocal mode, excitation was sourced from 488 $nm$ line from a multi-line laser system (Cairn Research, U.K.) and the emission signal was detected using a photomultiplier tube (Thorlabs, USA) with a source-blocking filter. Image analysis was performed using Fiji[3].

**Fluorescent Microsphere Uptake Assay;**
Multi-excitatory fluorescent microspheres (dia. 200 $nm$) were seeded at an approximate density of 1.14x10$^{10}$ beads/$ml$ with the JM105 inoculum and grown for 18 hours at 37°C in an imaging mould. Uptake was observed by widefield epi-fluorescence mesoscopy as above, but used dual-excitation and emission of the EGFP ($\lambda_{ex.}$ = 490 $nm$, $\lambda_{em.}$ = 512 $nm$) and the microspheres ($\lambda_{ex.}$ = 560 $nm$, $\lambda_{em.}$ = 612 $nm$).

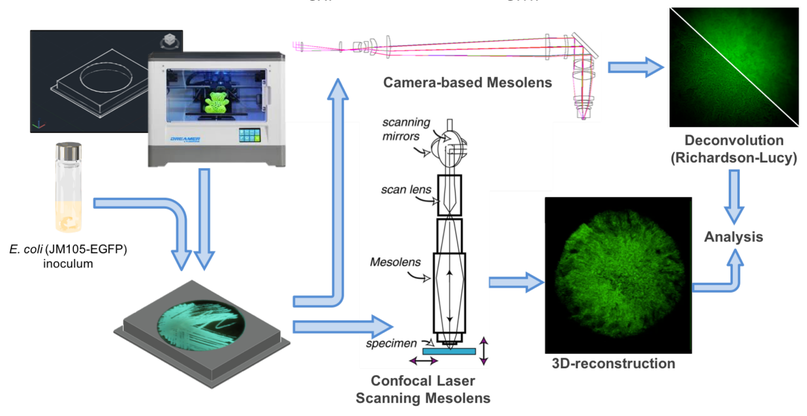
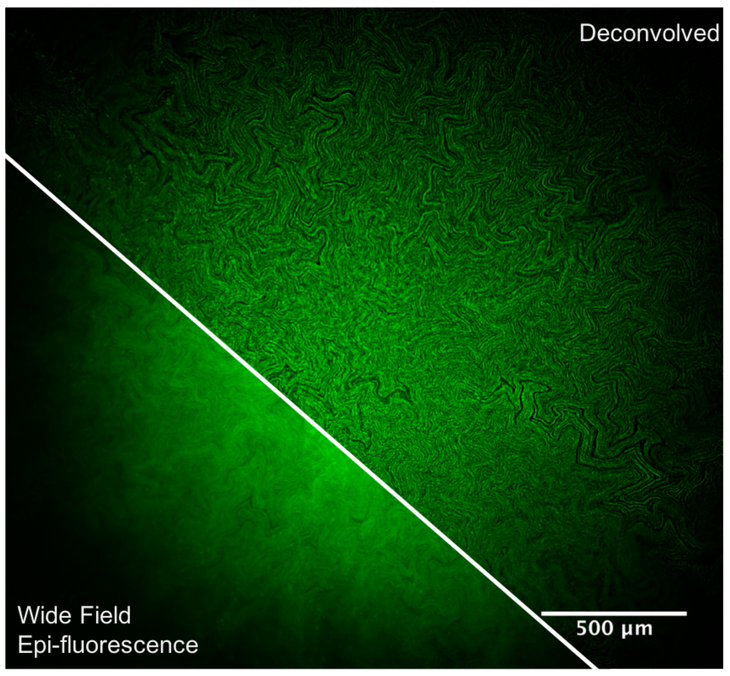



**Figure 1.** Flow diagram illustrating the methods used in this work. AutoCAD (Autodesk, USA) was used to design a 3D-printed chamber slide which could be used to grow colony biofilms. Widefield epi-fluorescence mesoscopy or confocal laser scanning mesoscopy was then used to assess the architecture of the colony biofilm.

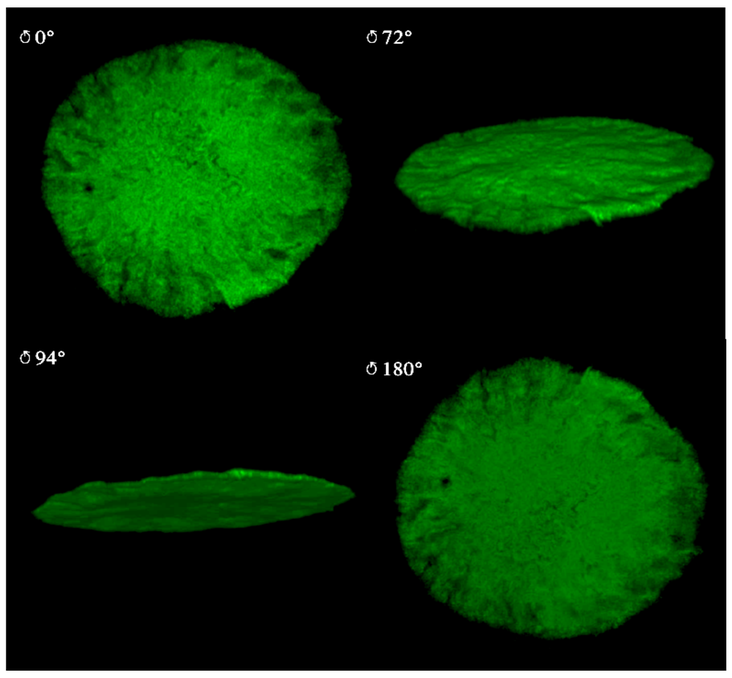
## Widefield Epi-fluorescence Mesoscopy




**Intra-colony channel systems are revealed by widefield epi-fluorescence mesoscopy**

**Figure 2.** Widefield epi-fluorescence mesoscopy revealed the previously undocumented internal architecture of colony biofilms. Applying a deconvolution algorithm to the raw data (*right*) improved the image quality and revealed a network of channel-like structures radiating throughout the biofilm. Each channel measures approximately 15 $\mu m$ wide.

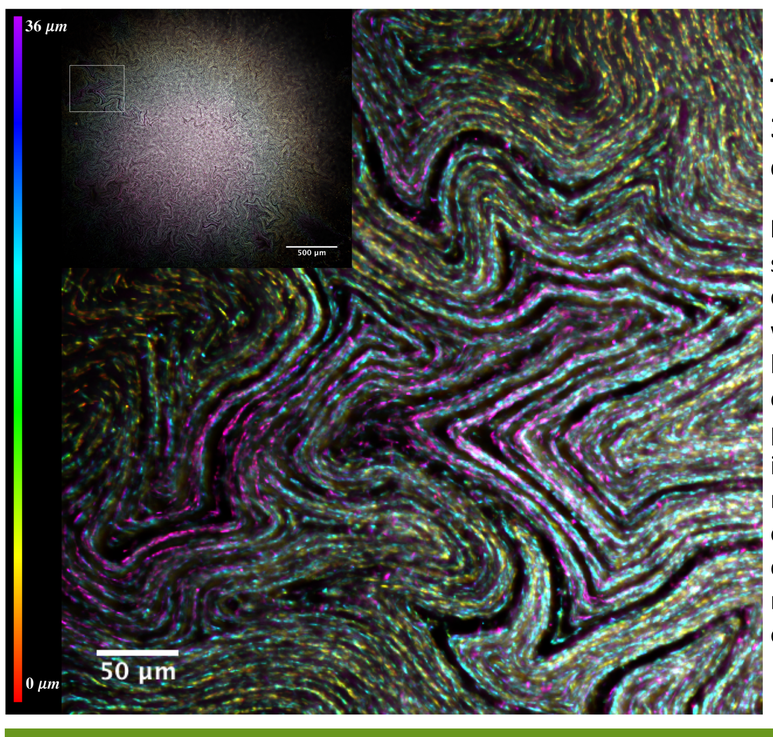
## 3D Reconstruction of an *E. coli* Colony Biofilm




**Confocal laser scanning mesoscopy concurs with the results of deconvolution of widefield data**

**Figure 3.** Three-dimensional reconstructions of colony biofilms can be generated using processed z-stacks of confocal laser scanning mesoscopy data. The arrangement of intra-colony channels agrees with observations in widefield mode, which shows that our deconvolution process does not introduce false spatial patterns as an artefact. Reconstruction shows that the channel systems permeate from the basal to the apical surface of these colonies. Diameter of colony shown is ca. 3 $mm$.

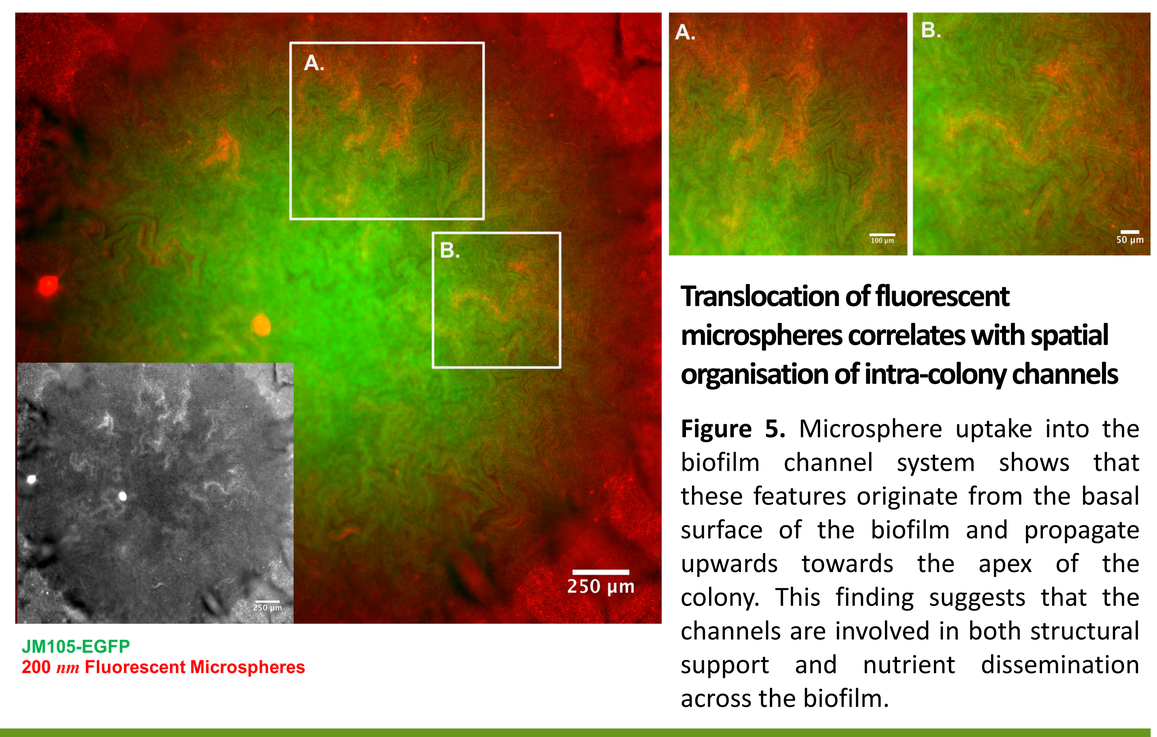
## Intracolony Channel-System Topography




**Topological mapping reveals 3D organisation of the intra-colony channel system**

**Figure 4.** A deconvolved 36 $\mu m$ sub-stack selected from the centre of a 150 $\mu m$ thick widefield z-stack of a colony biofilm. The applied colour map corresponds to the axial position of each optical section in the sub-stack. The magnified region reveals the 3D organisation of the biofilm channel system which appear as ridges protruding from the base of the colony.

## Microsphere Uptake Assay




**Translocation of fluorescent microspheres correlates with spatial organisation of intra-colony channels**

**Figure 5.** Microsphere uptake into the biofilm channel system shows that these features originate from the basal surface of the biofilm and propagate upwards towards the apex of the colony. This finding suggests that the channels are involved in both structural support and nutrient dissemination across the biofilm.

## Conclusions

- The Mesolens offers a novel imaging method for studying large microbial populations with sub-cellular resolution throughout the three-dimensional dataset.
- We have observed an intra-colony channel system which we suggest plays a role in both structural support and nutrient dissemination throughout the biofilm.

## References

[1] Percival. *et al*, 2015. *J. Med. Microbiol.*, **64**; [2] McConnell *et al*., 2016. *eLife*, **5**; [3] Schindelin *et al*., 2012. *Nat. Methods*, **9** (7)

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